Physical and mechanical properties of lactose/WPI mixtures: effect of pre-crystallisation

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ABSTRACT

This study investigated the physical and mechanical properties of spray-dried lactose/whey protein isolate (WPI) (4:1) mixtures with different contents of α -lactose monohydrate (1.0%, 11.2%, 29.2%, and 46.8%, w/w). Particle size of samples with 11.2%, 29.2%, and 46.8% crystallinity was significantly ($P < 0.05$) larger compared with the sample with 1.0% crystallinity. The presence of less than 46.8% crystalline lactose in lactose/WPI mixtures had only a minor effect on water sorption behaviour at a_w 0.11–0.44, whereas samples with higher crystallinity had higher stable water content after showing lactose crystallisation. Moreover, samples with lower crystallinity showed higher initial sorption rates. Increasing the amount of crystalline lactose had no significant influence on the glass transition temperature and the initial crystallisation temperatures at a_w 0.11–0.44. Furthermore, dairy powders with higher crystallinity had higher stiffness and water plasticisation showed a stronger effect on the structural relaxation of dairy powders with lower crystallinity.

1. Introduction

The main carbohydrate in dairy powders is lactose (Arellano, Aguilera, & Bouchon, 2004), which can exist in various crystalline and non-crystalline forms. The crystalline state is a solid state, having molecules well arranged in regular lattice. According to Haque and Roos (2005), the main types of crystallised lactose are α -lactose monohydrate, anhydrous β -lactose, and anhydrous forms of α - and β -lactose in molar ratios of 5:3 and 4:1. Compared with lactose in the crystalline state, the molecular arrangement of lactose in the amorphous state is disordered. Moreover, amorphous lactose is thermodynamically unstable and hygroscopic, easily absorbing moisture from the surroundings and then plasticising, while the crystalline lactose is thermodynamically stable and much less hygroscopic. These different state forms affect the behaviour of lactose and materials containing lactose, such as dairy powders (McSweeney & Fox, 2009). There has been considerable interest in amorphous components in dairy and pharmaceuticals industries. The presence of even small amounts of amorphous components can have a significant impact on the physico-chemical properties of materials, and subsequently alter product performance (Buckton & Darcy, 1996).

According to Bronlund and Paterson (2004) and Ibach and Kind (2007), the amorphous form of lactose has a much higher water content at a given humidity than the crystalline form and Hogan and Buckton (2001) stated that water sorption can be used to quantify the amorphous content of predominantly crystalline materials. In addition, Bronlund and Paterson (2004) indicated that the amount of water sorbed by crystalline powders at high water activity was dependent on the packing density of the powder particles, and small quantities of amorphous lactose present on crystalline powders caused significant changes to the water sorption behaviour.

In dairy powders, amorphous solids have a liquid-like structure with an extremely high viscosity. The amorphous residue will be changed to a nonequilibrium liquid state when temperature increases. Under the same thermodynamic conditions amorphous solids display higher molecular mobility than crystalline solids (Liu, Bhandari, & Zhou, 2006). The molecular mobility of the amorphous components is of great importance in determining the physical stability of food materials (Hancock, Shamblin, & Zografi, 1995). When the temperature increases from below to above the glass transition temperature (T_g), many of the physical properties of the amorphous materials show a rapid change, including increases in the free volume, molecular mobility, and dielectric coefficient. Additionally, large changes in viscoelastic properties of amorphous powders were expected above T_g (Jones, 1999; Kasapis, 2001; Royall et al., 2005). Crystallisation of amorphous components in food products may enhance both physical and chemical stability (Berlin, Anderson, & Pallansch, 1968a, 1968b; Miao & Roos, 2004; Roos & Karel, 1991), and changing the crystallinity of lactose in dairy powders often affects product quality (Aguilar & Ziegler, 1994; Roos, Jouppila, & Söderholm, 1999).

Water sorption behaviour, crystallisation and glass transitions of amorphous lactose, amorphous lactose/protein systems and amorphous lactose/salts mixtures after freeze-drying or spray drying have been widely studied (Bandyopadhyay, Das, & Sharma, 1987; Berlin, Anderson, & Pallansch, 1968a,b, 1970; Foster, Bronlund, & Paterson, 2005; Haque & Roos, 2004a,b, 2005, 2006; Haque, Kawai, & Suzuki, 2006; Hermansson, 1977; Hogan & Buckton, 2001; Jouppila, Kansikas, & Roos, 1997; Kaminaski & Al-Bezweni, 1994; Potes, Kerry, & Roos, 2012; Shrestha, Howes, Adhikari, & Bhandari 2007). Moreover, the amounts of crystalline lactose can affect the sorption behaviour of lactose powders significantly (Bronlund & Paterson, 2004). However, there has been no further study about the effects of crystalline lactose content on mechanical properties of dairy powders, which is essential for

dairy research and industry. The objectives of this study were to determine the influence of crystalline lactose content on physical and mechanical properties of lactose/ whey protein isolate (WPI) mixtures.

2. Materials and methods

2.1. Materials

Powdered α -lactose monohydrate (> 99% purity) was kindly donated by Arla Foods Ingredients (Viby J, Denmark). WPI, containing 71% β -lactoglobulin and 12% α -lactalbumin, was obtained from Davisco Food International (Le Sueur, MN, USA). Aluminium oxide calcined powder was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sample preparation

Lactose/WPI (4:1) mixtures at 40% (w/w) dry matter were prepared in de-ionised water at 65 °C in a water bath for 2 h with a stirring speed of 500 rpm. These solutions were kept at room temperature (20–22 °C) with a stirring speed of 150 rpm for 0, 3, 15 and 20 h pre-crystallisation; these lactose/WPI mixtures were defined as S1, S2, S3 and S4, respectively, according to the pre-crystallisation time. After pre-crystallisation, samples were spray-dried using an ANHYDRO spray dryer with a centrifugal atomiser (Copenhagen, Denmark). The inlet air temperature was around 170 °C, and the outlet temperature was around 90 °C. Spray-dried solids were immediately placed in evacuated desiccators over P₂O₅ at room temperature. Each analysis was carried out within 3 months after spray-drying.

2.3. Powder characterisation

2.3.1. Determination of α -lactose monohydrate content in spray-dried lactose/WPI mixtures

The content of α -lactose monohydrate in spray-dried lactose/WPI mixtures was determined according to the method of Schuck and Dolivet (2002) based on the difference between total water, determined using a Karl Fischer Titration (Mettler Toledo International Inc., Im Langacher Greifensee, Switzerland), and unbound water, determined by weight loss following 6 h drying of 1 g powder at 87 °C. In this study, the content of α -lactose monohydrate was used to represent the crystallinity of lactose/WPI mixtures. It was assumed that there was no bound water in amorphous lactose. Each analysis was carried out in triplicate.

2.3.2. Light microscopy

Lactose crystals were observed with a polarised light microscope fitted with a ProgRes® camera system (JENOPTIK I Optical Systems, Jena, Germany). The lactose/WPI mixtures were spread on slides and one drop of sunflower oil was used to blend with the samples. Coverslips were then placed on the sample, which was observed with a 10× objective. Images were acquired using an Olympus BX51 light microscope (Olympus Optical Co. Ltd., Tokyo, Japan) with polariser filters.

2.3.3. Powder characteristics

Protein content was determined using a FP 628 Nitrogen Determinator (LECO Corporation, Lakeview Avenue, St. Joseph, MI, USA). Lactose content was determined using an Automatic Polarimeter (Autopol 1, Rudolph Research Analytical, Hackettstown, NJ, USA). Water content was determined using a HR83 Hologen Moisture Analyzer (Mettler Toledo International Inc., Im Langacher Greifensee, Switzerland). All chemical analysis of

powders was carried out immediately after manufacture. Powder particle size distribution and specific surface area (SSA) were determined by laser light scattering using a Malvern Mastersizer 3000 (Malvern Instruments, Worcestershire, UK).

2.4. Water sorption and lactose crystallisation

Water sorption for each powder was measured using the static gravimetric method and performed in triplicate. Approximately 1 g of spray-dried lactose/WPI mixtures was weighed into small glass vials (25 mL) and dried in a vacuum oven (OV-12, Meline Industries, Inc., Mundelein, IL, USA) at 45 °C for 48 h to remove residual water. After drying, all samples were equilibrated for 240 h in evacuated desiccators over different saturated salt solutions. These saturated salt solutions were LiCl, CH₃COOK, MgCl₂, K₂CO₃, Mg(NO₃)₂, and NaNO₂, which gave relative humidity (RH) of 11%, 23%, 33%, 44%, 54%, and 65%, respectively. All desiccators were placed in incubators set at 25 °C during equilibration. The samples were weighed at 0, 3, 6, 9, 12 and 24 h, and then at 24 h intervals. Water content of each powder was measured as a function of time and the mean weight of triplicate samples was calculated.

The water sorption of lactose/WPI mixtures at 24 h was also monitored using a Multisample Dynamic Moisture Sorption SPS11-10 μ (ProUmid GmbH & Co. KG, August-Nagel-Str. 23, Ulm, Germany). The measurement cycles were started at 11%, 23%, 33%, 44%, 54%, and 65% RH, respectively, and ended at 11%, 23%, 33%, 44%, 54%, and 65% RH, respectively. The time for one cycle was 24 h. The measurement temperature was 25 $\pm$ 0.1 °C. Sorption rate of spray-dried lactose/WPI mixtures was calculated according to the water sorption results:

$$\text{Sorption rate} = \text{Water content/time (\% h}^{-1}\text{)} \quad (1)$$

2.5. Differential scanning calorimetry

T_g (onset), and initial crystallisation temperatures, (T_{ic}) of lactose/WPI mixtures were determined using a differential scanning calorimeter (DSC Q2000, TA Instruments, Crawley, UK). To determine the T_g , dairy powders (1 g) were transferred to glass vials and dried in the vacuum oven at 45 °C for 48 h. The dehydrated powders were equilibrated in evacuated desiccators over P_2O_5 and saturated salt solutions of LiCl, CH_3COOK , $MgCl_2$, and K_2CO_3 for 168 h. Then 10 to 15 mg of equilibrated powders was transferred to Tzero pans (TA Instruments). The DSC pans were hermetically sealed with Tzero hermetic lids and samples were analysed. An empty pan was used as a reference. At the first scan, the samples were heated to at least 20 °C higher than the glass transition temperature region at 5 °C min⁻¹ and then cooled at 10 °C min⁻¹ to 0 °C; a second heating scan was then run to at least 100 °C higher than the glass transition temperature at 5 °C min⁻¹. Anhydrous samples were scanned using pans with punctured lids to allow evaporation of residual water during the measurements (Silalai & Roos, 2010). At the first scan, the samples were heated at 5 °C min⁻¹ to 100 °C to evaporate residual water and then cooled at 10 °C min⁻¹ to below glass transition. A second heating scan was then performed on the anhydrous powders to far above the glass transition temperature at 5 °C min⁻¹. All measurements were carried out in duplicate. T_g values were taken as the onset-point of the endothermic baseline shift. T_g and T_{ic} were determined using TA Universal analysis software, version 5.1.2 (TA Instruments).

2.6. Dynamic mechanical analysis

A Dynamic Mechanical Analyser (DMA Q800, TA Instruments) was used, in conjunction with a gas cooling accessory (GCA) tank, to determine the dynamic mechanical

properties of lactose/WPI mixtures. A rectangular stainless steel powder holder was designed to generate a defined geometry to contain powder with inner dimensions of 60 mm × 11 mm × 1 mm. A pre-weighed mass of powder mixed with aluminium oxide calcined powder at the ratio 4:1 was evenly spread within this shallow container, and the upper lid was then placed onto the top surface of the powder (Mahlin, Wood, Hawkins, Mahey, & Royall, 2009). As aluminium oxide calcined powder showed no influence on mechanical property results of dairy powders in the temperature range, it was added to protect dairy powder from sticking on the powder holder during the heating test. The sample holder was mounted in the instrument in a dual cantilever clamp during measurement. For anhydrous samples and samples equilibrated at a_w 0.11, the measurements were made at a heating rate of 2 °C min⁻¹ from 0 to 150 °C. For samples equilibrated at a_w 0.23, 0.33 and 0.44, the measurements were made at a heating rate of 2 °C min⁻¹ from 0 to 120 °C. DMA was operated by a sinusoidal deformation applied to the powder sample holder at a fixed strain. The amplitude was 15 µm. During dynamic heating, the samples were analysed for storage modulus (E') and loss modulus (E'') using single frequency 1 Hz. Frequency of deformation (oscillating frequency) was changed in multiple frequencies of 0.5, 1, 3, 5, 10, and 20 Hz. The α -relaxation times were determined directly from the frequencies (f) using the relationship of Eq. (2) (Noel, Parker, & Ring, 2000):

$$\tau_{\alpha}=1/(2\pi f) \quad (2)$$

The temperature dependence of α -relaxation time was modelled using the Vogel-Tamman-Fulcher (VTF) relationship of Eq. (3) (Angell, 2008; Silalai & Roos, 2011a):

$$\tau_{\alpha}=A\exp[DT_0/(T-T_0)] \quad (3)$$

where A, D and T_0 are constants.

2.7. Statistical analysis

Measurement of protein content, glass transition, and dynamic mechanical analysis were performed in duplicate, with all other analyses performed in triplicate. Results were expressed as mean $\pm$ standard deviations (SD). One-way analysis of variance (ANOVA), followed by Tukey's test, was used to determine the significant differences between the mean values of each test (Microsoft Office Excel 2010, Microsoft, Inc., Redmond, WA, USA). A significance level of $P < 0.05$ was used throughout the study.

3. Results and discussion

3.1. Powder characterisation

The characteristics of lactose/WPI mixtures are shown in Table 1. Crystalline lactose content increased as the pre-crystallisation time was increased (Table 1), yielding samples containing 1.0% (S1), 11.2% (S2), 29.2% (S3) and 46.8% (S4) crystalline lactose. The amount of crystalline lactose is defined as crystallinity of the samples in this study.

The physical appearance of samples is shown in Fig. 1. It was obvious that the amount of crystalline lactose increased from S1 to S4, which further confirmed that the crystallinity of S1, S2, S3 and S4 was different. Lactose crystals were very hard and brittle and its particle shape was different from that of amorphous lactose, which might affect the physical properties and flow properties of dairy powders.

Protein and lactose contents for lactose/WPI mixtures were around 20% and 80%, respectively (Table 1). The particle size of S2, S3, and S4 was significantly larger than S1. However, there were only minor differences in the particle size between samples S2, S3, and S4. As specific surface area (SSA) values are typically inferred from particle size data, S1

with the smallest particle size showed the largest SSA value. These results indicated that pre-crystallisation before spray drying could increase the particle size of dairy powders. But the amount of crystalline lactose showed only a minor effect on the particle size of powders. These differences in particle size and SSA values of dairy powders can affect their physical properties and functionality.

3.2. Water sorption and lactose crystallisation

3.2.1. Water sorption

The experimental water content of lactose/WPI mixtures with different crystallinity equilibration at various RH is shown in Table 2. In lactose/WPI mixtures, the water may be sorbed by proteins, amorphous lactose, crystalline lactose, with some bound as the water of crystallisation 5% of mass of crystals. The amount of water sorbed by S2 with 11.2% crystallinity was significantly higher ($P < 0.05$) than those of S1 (1.0% crystallinity), S3 (29.2% crystallinity), and S4 (46.8% crystallinity) at water activity range of a_w 0.11- 0.33, while S3 sorbed the highest amount of water at a_w 0.44. S1, with the lowest crystallinity, did not sorb the highest amount of water at water activity range of a_w 0.11- 0.44, but did S1 sorb the highest amount of water at a_w 0.54 and 0.65 before showing loss of sorbed water. According to Bronlund and Paterson (2004), crystalline lactose sorbed very little water over the water activity range (a_w 0-0.85). However, the presence of crystalline lactose in lactose/WPI mixtures did not show significant effect on their water sorption behaviour during equilibration (Table 2). It was assumed that the presence of WPI in dairy powders weakened the effect of crystalline lactose on water sorption behaviour of lactose/WPI mixtures. Previous studies also indicated that water contents sorbed by lactose/WPI mixtures were determined by water sorption behaviour of non-crystalline lactose and protein (Fan & Roos 2015).

Sorbed water of lactose/WPI mixtures decreased within 24 h at $a_w > 0.44$ as a result of lactose crystallisation (Fig. 2). Loss of sorbed water occurred more rapidly at $a_w 0.65$ as compared with at $a_w 0.54$, which was in agreement with the study of Haque and Roos (2004a). Since loss of sorbed water does not occur in crystalline lactose, S2, S3, and S4 showed higher amount of stable water after lactose crystallisation than those of S1 without pre-crystallisation at $a_w 0.54$ and 0.65 (Fig. 2). These results indicated that the crystallinity showed minor influence on the water sorption behaviour of dairy powders containing protein at $a_w 0.11$ – 0.44 . However, dairy powders with higher crystallinity showed higher stable water content after lactose crystallisation.

3.2.2. Sorption rate

Water sorption behaviour of lactose/WPI mixtures with different crystallinity was also measured using a Multisample Dynamic Moisture Sorption SPS11-10 μ . Water sorption of lactose/WPI mixtures were near equilibrium within 24 h (raw data not shown). Using the water sorption results, sorption rates in the first hour of equilibration were calculated (Fig. 3). The sorption rates of lactose/WPI mixtures increased with increasing storage relative humidity (Fig. 3A–F). The sorption rates of lactose/WPI mixtures decreased and then arrived at constant values (Fig. 3), and the final constant sorption rates of S1, S2, S3 and S4 were not significantly different at $a_w 0.11$ (Fig. 3A). The sorption rates of S2, with 11.2% crystallinity, were higher than those of S1, S3, and S4 at $a_w 0.23$ – 0.65 . In addition, the initial sorption rates of lactose/WPI mixtures were divided into two groups, higher initial sorption rate group for S1 and S2 with lower crystallinity, and lower initial sorption rate group for S3 and S4 with higher crystallinity (Fig. 3). It was obvious that the difference between two groups increased with increasing storage relative humidity. Therefore, the crystallinity affected the initial

sorption rate of the lactose/WPI mixtures significantly; samples with higher crystallinity showed lower sorption rates.

3.3. Glass transition

The T_g and T_{ic} values for anhydrous and equilibrated lactose/WPI mixtures are shown in Table 3. It was obvious that the T_g values of lactose/WPI mixtures decreased with increasing water content, which showed typical water plasticisation of dairy powders containing lactose (Haque et al., 2006; Jouppila et al., 1997; Potes et al., 2012). There was no significant difference in the T_g values of lactose/WPI mixtures with different levels of crystallinity at the same water activities. Therefore, the T_g values of lactose/WPI mixtures with different amount of crystalline lactose was primarily dependent on the amorphous lactose, which was in agreement with previous studies. According to previous studies (Fan & Roos, 2015; Potes et al., 2012; Silalai & Roos, 2010), lactose-protein systems showed phase separation and their T_g values were dependent on amorphous lactose. The T_g values of lactose/WPI mixtures in this study were higher than those reported by Haque and Roos (2004b) and Fan and Roos (2015) for lactose/WPI mixtures at corresponding water activities. As water content significantly affected T_g values of dairy powders, the differences in the T_g values between our study and other studies were probably a result of different water contents with equilibration at a_w 0.11–0.44. Haque and Roos (2004a) have indicated that drying methods, storage time, and storage temperature could result in the differences in final water content of lactose/protein mixtures. In addition, the T_{ic} values of lactose/WPI mixtures also decreased with increasing water content (Table 3). Since lactose crystallisation could be delayed by the presence of protein, the T_{ic} values of lactose/WPI mixtures was higher than that of pure lactose (Haque & Roos, 2004b; Zhou & Roos, 2012). Lactose/WPI mixtures with

different amount of crystalline lactose showed similar T_{ic} values in the range of a_w 0.11-0.44. Therefore, the amount of crystalline lactose showed no significant influence on the T_{ic} values of the lactose/WPI mixtures.

3.4. Mechanical properties

3.4.1. Modulus and stiffness

The physical properties of amorphous materials often change around the glass transition temperatures, which may result in an α -relaxation (Roudaut, Simatos, Champion, Contreras-Lopez & Le Meste, 2004; Silalai & Roos, 2011a,b). The mechanical properties of lactose/WPI mixtures with different amount of crystalline lactose were measured by DMA. A loss of water occurred during dynamic measurements for dairy powders with lower water activities ($a_w < 0.23$), which was also reported by Silalai and Roos (2011b). Moreover, lactose/WPI mixtures showed lactose crystallisation during storage at a_w 0.54 and 0.65. Therefore, the results of mechanical properties of lactose/WPI mixtures at a_w 0.11, 0.54 and 0.65 are not shown. Mechanical α -relaxation of lactose/WPI mixtures occurred above T_g and was observed from a sharp decrease in their storage modulus and a peak in their loss modulus (Fig. 4). The storage modulus shows the amount of energy that the material stores (Edward, Kirui, Omolo, Ngumbu & Odhiambo, 2014). Furthermore, the magnitudes of storage modulus and loss modulus changes are relative to molecular mobility (Roudaut et al., 2004; Silalai & Roos, 2010, 2011b). As can be seen from Fig.4 A1, B1, and C1, samples S4, with the highest crystallinity, showed higher storage modulus than samples S1, S2, and S3 at the glassy state at a_w 0.23–0.44. Additionally, the difference in storage modulus of lactose/WPI mixtures increased with increasing storage RH (Fig. 4 A1, B1 and C1). At the glassy state, the storage modulus of lactose/WPI mixtures with lower crystallinity (S1 and S2) decreased

significantly from a_w 0.33 to a_w 0.44 (from 208,295 MPa to 135,679 MPa for S1; from 214,528 MPa to 110,495 MPa for S2) (Fig. 4 B1 and C1). The storage modulus of S4 with 46.8% crystallinity showed the smallest change at the glassy state with increasing water content (222,785 MPa at a_w 0.33; 214,190 MPa at a_w 0.44). These results indicated that the addition of crystalline lactose could maintain the stiffness of dairy powders when water content is increased. This might be due to the different molecular mobility and free volume of amorphous and crystalline state lactose. According to Kilburn et al. (2004), the effect of water plasticisation in carbohydrates solids is related to hydrogen bond formation and disruption, and also involves to the changes in free volume. Unlike amorphous structure, the crystalline state has molecules well arranged, which is in the favourable low energy state. Water plasticisation showed no significant effect on molecular mobility of crystalline lactose, and only exhibited a minor effect on the storage modulus of dairy powders with higher crystallinity. At the glass transition region, the storage modulus of lactose/WPI mixtures decreased significantly at a_w 0.23, 0.33 and 0.44 as a result of increasing molecular mobility (Fig. 4 A1, B1 and C1). A greater change in the storage modulus was experienced with lactose/WPI mixtures at a_w 0.33 and 0.44 compared with at a_w 0.23, which indicated that lactose/WPI mixtures with higher water content showed higher molecular mobility at the glass transition region. Therefore, the magnitude of the storage modulus changes of lactose/WPI mixtures were affected by the amount of crystalline lactose and water. Dairy powders with higher crystallinity showed lower molecular mobility with increasing temperature.

Stiffness of materials refers to the ability to carry stress without changing dimension (Ebewele, 2000). For the measurement of unconstrained uniaxial tension or compression, Young's modulus can be used as a measure of the stiffness of a material. In this study, the change of storage modulus could reflect the change of stiffness of lactose/WPI mixtures. The

changes of stiffness for lactose/WPI mixtures were directly related to the storage modulus when temperature increased from 0 to 120 °C at 1 Hz (the stiffness results were not shown). S4 with the highest crystallinity gave the largest storage modulus at a_w 0.23, 0.33 and 0.44 (Fig. 4 A1, B1 and C1), which meant S4 had the highest stiffness at a_w 0.23 to 0.44. However, S1 and S2 with lower crystallinity showed lower stiffness at a_w 0.33 and 0.44. These results indicated that dairy powders with higher amount of crystalline lactose had higher stiffness, which might be due to the physical properties of crystalline lactose. As crystallinity is the result of a highly ordered arrangement of the lactose molecules, lactose crystals are very hard and stiff.

The changes of loss modulus for lactose/WPI mixtures with different crystallinity were also determined using the DMA. The loss modulus, E'' , of lactose/WPI mixtures showed minor changes at the glassy state and the rubbery state, while they increased dramatically and arrived at the peak values in the glass transition region (Fig. 4 A2, B2 and C2). This result could be explained from the point of mechanical internal friction (Guo, Feng, Wang, Hu, & Zhang, 2013). According to Guo et al. (2013), the chain segment motion of lactose and proteins molecules is in a frozen state when in the glassy state, and is in a free moving state when in the rubbery state. The chain segment motion changes from a frozen state to a free moving state during the glass transition region. This change of motion leads to large internal loss. The internal friction loss approaches maximum as the temperature reaches glass transition temperature, which is reflected from the peak of loss modulus. Since the physical state of amorphous powders is strongly affected by the concentration of water, the peak values of loss modulus for lactose/WPI mixtures increased as water content was increased (Fig. 4 A2, B2, and C2). Moreover, the loss modulus of lactose/WPI mixtures in the glassy state also increased with increasing water content, especially for S1 with the lowest crystallinity (3211 MPa at a_w 0.33; 11,442 MPa at a_w 0.44). Since the magnitude of loss

modulus changes is also related to the molecular mobility, dairy powders with lower crystallinity showed higher molecular mobility as water content was increased.

3.4.2. Mechanical structural relaxation

In this study, the α -relaxation temperatures, T_α , were taken from the temperatures of loss modulus peak values at 1 Hz. T_α values of dairy powders shifted to lower temperatures with increasing water content (Table 3), which was in agreement with previous studies (Li, Roos, & Miao, 2016; Potes et al., 2012; Silalai & Roos, 2011b). Lactose/WPI mixtures with different crystallinity showed similar T_α values at a_w 0 and 0.11, while S1 with the lowest amount of crystalline lactose showed the highest T_α values at a_w 0.23-0.44. Furthermore, the mechanical α -relaxations for lactose/WPI mixtures at a_w 0.23, 0.33, and 0.44 were determined by DMA using multi-frequency mode. The magnitude of mechanical α -relaxations for lactose/WPI mixtures decreased when frequency was increased (data not shown).

Many studies have indicated that the Vogel-Tammann-Fulcher (VTF) relationship can be used to describe the temperature dependence of relaxation times for amorphous materials at temperatures above glass transition (Angell, 1995, 2008; Angell, Ngai, McKenna, McMillan, & Martin, 2000; Potes et al., 2012; Silalai & Roos, 2011b). The VTF plots indicated that the T_α values of lactose/WPI mixtures at various frequencies were at higher temperatures with increasing frequency (Fig. 5). Moreover, lower $T-T_g$ values were shown by S1 for the corresponding relaxation at a_w 0.23-0.44, which meant α -relaxation of S1 occurred at lower temperatures above T_g values than those of S2, S3, and S4. Comparing the VTF plots at a_w 0.23 and 0.33 (Fig. 5 A and B), the α -relaxations of lactose/WPI mixtures occurred at higher $T-T_g$ values as water content was increased. Then the $T-T_g$ values of S1 and S2 decreased when a_w was increased from 0.33 to 0.44 (Fig. 5 B and C), while those of S3 and

S4 showed only minor changes. According to Liu et al. (2006), the viscosity and molecular mobility of S1 and S2 with lower crystallinity might have strong temperature dependence. Thus, they exhibit larger changes on relaxation time in the vicinity of the glass transition than dairy powders with higher crystallinity. Therefore, water plasticisation had a stronger effect on the structural relaxation of dairy powders with lower crystallinity.

4. Conclusions

As crystalline lactose and amorphous lactose have different molecular arrangement, they show different behaviour in physical properties and functionality. Lactose/WPI mixtures with different amount of crystalline lactose were prepared by pre-crystallisation. Particle size of dairy powders with 11.2%, 29.2%, and 46.8% crystallinity was significantly ($P < 0.05$) larger than that of dairy powder with 1.0% crystallinity. Since physical properties, flowability and functionality of dairy powders are highly dependent on the particle size of powders, pre-crystallisation could have a significant impact on the final properties of dairy powders. The presence of less than 46.8% crystalline lactose in dairy powders showed only a minor effect on water sorption behaviour of dairy powder at a_w 0.11–0.44. Dairy powders with higher crystallinity showed higher stable water content after lactose crystallisation. Furthermore, dairy powders with lower amount of crystalline lactose showed higher sorption rates at the beginning of water sorption. Increasing the amount of crystalline lactose in dairy powders did not have significant influence on the T_g and T_{ic} values. The mechanical property study showed that lactose/WPI mixtures with higher amount of crystalline lactose had higher stiffness, and the addition of crystalline lactose could maintain the stiffness of dairy powders when water content is increased. Water plasticisation had a stronger effect on the structural relaxation of dairy powders with lower amount of crystalline lactose. Since pre-crystallisation

of lactose is widely used in the production of dairy powders, the findings of this study could be very useful in the dairy industry.

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1 **Table 1**

2 Powder characteristics of lactose/WPI (4:1) mixtures. ^a

System	Crystallinity (%)	Protein content (%)	Lactose content (%)	Water content (%)	d_{50} (μm)	Specific surface area (m ² kg ⁻¹)
S1	1.0±0.58	19.94±0.07	78.91±0.02	2.47±0.04	22.85±0.25 ^c	714.75±7.45 ^a
S2	11.2±0.97	19.55±0.04	79.03±0.11	2.93±0.16	25.35±0.05 ^a	629.95±0.85 ^c
S3	29.2±0.92	19.45±0.02	78.99±0.06	2.45±0.09	25.20±1.20 ^{ab}	682.10±9.30 ^b
S4	46.8±1.11	19.66±0.02	79.45±0.09	2.53±0.15	23.85±0.05 ^b	695.05±1.05 ^b

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4 ^a Values are means ± standard deviation (n=2 for protein content; for the other values, n=3);
5 values within columns with different superscript letters are significantly different at $P < 0.05$.

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Table 2

Water content of lactose/WPI (4:1) mixtures with 1.0% (S1), 11.2% (S2), 29.2% (S3) and 46.8% (S4) crystallinity after equilibration at a_w 0.11–0.65 at 25 °C for 240 h. ^a

a_w	S1	S2	S3	S4
0.11	2.48±0.00 ^b	2.77±0.03 ^a	2.54±0.09 ^b	2.45±0.03 ^b
0.23	4.13±0.11 ^b	4.33±0.05 ^a	4.26±0.04 ^a	4.00±0.04 ^c
0.33	5.94±0.11 ^b	6.05±0.05 ^a	6.04±0.07 ^a	5.71±0.05 ^c
0.44	8.69±0.07 ^b	8.52±0.02 ^c	8.76±0.11 ^a	8.43±0.05 ^d
0.54	5.68±0.04 ^b	6.78±0.32 ^a	6.85±0.07 ^a	6.90±0.06 ^a
0.65	7.17±0.08 ^c	7.78±0.13 ^b	7.85±0.06 ^b	8.51±0.05 ^a

^a Values (g 100 g⁻¹ of dry solids) are means ± standard deviation (n=3); values within columns with different superscript letters are significantly different at $P < 0.05$.

Table 3

Glass transition temperature (T_g), initial crystallisation temperature (T_{ic}), and α -relaxation temperature (T_α) of lactose/WPI (4:1) mixtures with 1.0% (S1), 11.2% (S2), 29.2% (S3) and 46.8% (S4) crystallinity equilibrated at a_w 0–0.44 at 25 °C for 168 h. ^a

Systems		Water activity (a_w)				
		0	0.11	0.23	0.33	0.44
S1	T_g	105±0.5	71±0.0	58±0.5	40±0.0	21±0.5
	T_{ic}	/	127±1.0	115±0.5	94±0.5	72±0.0
	T_α	126.5±0.4	126.6±0.4	80.0±0.3	70.6±0.9	44.4±1.1
S2	T_g	105±0.0	70±0.0	58±0.5	40±0.5	22±0.0
	T_{ic}	/	125±0.5	112±0.0	92±0.5	73±0.5
	T_α	125.8±0.1	125.1±0.3	77.8±0.7	66.4±0.9	37.4±0.9
S3	T_g	104±0.5	70±0.0	58±1.0	39±0.0	21±0.0
	T_{ic}	/	126±1.0	114±0.5	93±0.0	73±0.5
	T_α	127.0±0.3	127.0±0.3	78.9±0.3	61.0±0.6	38.0±0.9
S4	T_g	105±0.0	70±0.5	58±0.0	38±0.5	21±0.5
	T_{ic}	/	127±0.5	113±0.5	93±0.5	72±0.0
	T_α	127.0±0.4	126.3±0.2	77.9±0.5	62.0±0.8	41.0±0.9

^a Values are mean ± standard deviation (n=2).

Figure legends

Fig. 1. Images obtained by polarised light microscope for lactose/WPI (4:1) mixtures with 1.0% (■, S1), 11.2% (●, S2), 29.2% (▲, S3) and 46.8% (▼, S4) crystallinity.

Fig. 2. Lactose crystallisation at (A) a_w 0.54 and (B) a_w 0.65 at 25 °C of lactose/WPI (4:1) mixtures with 1.0% (S1), 11.2% (S2), 29.2% (S3) and 46.8% (S4) crystallinity.

Fig. 3. Sorption rate of lactose/WPI (4:1) mixtures with 1.0% (solid line, S1), 11.2% (dashed line, S2), 29.2% (dotted line, S3) and 46.8% (dot-dash line, S4) crystallinity during equilibration for 24 h at 25 °C at a_w of: A, 0.11; B, 0.23; C, 0.33; D, 0.44; E, 0.54; F, 0.65.

Fig. 4. Storage modulus (A, C, E) and loss modulus (B, D, F) of lactose/WPI (4:1) mixtures with 1.0% (dashed line, S1), 11.2% (dotted line, S2), 29.2% (dash-dot line, S3) and 46.8% (dash-dot-dot line, S4) crystallinity during equilibration at 25 °C for 168 h at a_w of: A and B, 0.23; C and D, 0.33; E and F, 0.44.

Fig. 5. Plots of $\ln$ relaxation time against temperature difference $1/(T-T_g)$ for lactose/WPI (4:1) mixtures with 1.0% (■, S1), 11.2% (●, S2), 29.2% (▲, S3) and 46.8% (▼, S4) crystallinity during equilibration at 25 °C for 168 h at a_w of: A, 0.23; B, 0.33; C, 0.44.

Figure 1

Figure 1

Figure 2

Figure 2

Figure 3

Figure 3

Figure 4

Figure 4

Figure 5

A

B

C

Figure 5